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Supplemental risk evaluations and status of *Puccinia carduorum* for biological control of musk thistle

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Abstract

Tests of seven rare and endangered native North American *Cirsium* species and four modern artichoke lines were requested in response to a proposal for introduction of *Puccinia carduorum* into the United States for biological control of musk thistle (*Carduus nutans* ssp. *leiophyllus*). These tests were supplemental to an earlier extensive host-range study that established *P. carduorum* from musk thistle as host specific, useful for biological control, and suitable for limited field tests in Virginia. Test plants in the current study were evaluated in support of a proposal to use the rust in the western United States, and particularly, in California. None of the test plants in this study had been evaluated in previous assessments and all were either rare, endangered or threatened in California. Tests were conducted in both field and greenhouse settings. Field tests were run for two seasons, and test plants were inoculated by natural spread of the pathogen from source plants inside rings of test plants. Greenhouse tests involved direct inoculation under optimal conditions of dew and temperature (18–20 °C, 16 h) for infection. None of the seven *Cirsium* species or subspecies tested became infected by *P. carduorum*, either in field or greenhouse tests, compared to infection of 98% of the individual musk thistle plants ($n = 102$) from all the studies. Modern artichoke cultivars were tested only by direct inoculation under optimal greenhouse conditions. All artichoke plants ($n = 115$) either were immune (no macroscopic symptoms, $n = 69$) or at most, resistant ($n = 46$); pustules on all but two of the resistant plants were very small ($\leqslant$0.30 mm diam). Despite infections on artichokes, *P. carduorum* could not be maintained on artichokes under optimal greenhouse conditions. These results confirm earlier findings from host-range tests and risk assessments of *P. carduorum*. This information suggests that rare, threatened, or endangered *Cirsium* spp. and modern artichoke cultivars are not likely to be adversely affected by the use of *P. carduorum* for biological control of musk thistle. These data have been reviewed by grower groups and regulatory agencies in a proposal for permission to use the rust for musk thistle control throughout the United States.
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1. Introduction

Puccinia carduorum Jacky is a candidate for biological control of musk thistle, *Carduus nutans* L. ssp. *leiophyllus* [Petrovic] Stoj. & Stef. (= *Carduus thoermeri* Weinm.) in the United States (US). Following extensive risk analyses under greenhouse and field conditions (Baudoin et al., 1993; Bruckart et al., 1996a; Politis et al., 1984), a proposal was made to APHIS in 1990 for permission to release the rust fungus in California. The proposal to use *P. carduorum* was reviewed by the United States Department of the Interior (USDI), Fish & Wildlife Service (F&WS) as part of the "NEPA process" which, under the National Environmental Policy Act (NEPA), authorizes the F&WS to review issues involving significant federal actions that may affect the

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environment. F&WS is responsible, in particular, for rare or otherwise endangered species. Recommendations by F&WS included evaluation of additional "Endangered" or "Threatened" (E/T) *Cirsium* spp. (US Fish & Wildlife Service, 2000) from the Western US that were not inoculated in earlier tests. *Cirsium pitcheri* Torr. (T&G), the only E/T species tested in the original risk analysis, was exempted because of concurrence by Region 3 (Great Lakes–Big Rivers Region which includes Illinois, Indiana, Iowa, Michigan, Missouri, Minnesota, Ohio, and Wisconsin) of the F&WS following review of data from the original proposal. Species of *Cirsium* considered rare in California (California Native Plant Society, 2001) also were recommended for testing.

The proposal to use *P. carduorum* was reviewed also by representatives of the California artichoke industry. This was at the request of the state of California, before issuing a permit for *P. carduorum*. This was particularly important, because artichoke culture has changed since initiation of the *P. carduorum* evaluation in the early 1980s. Some California growers now plant artichoke fields on an annual basis using seeds, compared with the long-established practice of growing artichokes as perennials from crowns. New artichoke cultivars, developed specifically for this modern artichoke production (Woods, D.M., California Department of Food and Agriculture [CDFA]; personal communication), had not been tested for susceptibility to *P. carduorum*.

This paper reports results from additional tests conducted to determine susceptibility of selected native, E/T *Cirsium* spp. and modern artichoke cultivars to *P. carduorum*, in response to recommendations of the F&WS and the artichoke industry. Data from these studies were originally reported to various regulatory agencies concerning the acceptability of *P. carduorum* for biological control of musk thistle in US and are not readily available to the scientific audience. Hence, we report these results here and review the status for use of *P. carduorum* for biological control of musk thistle in US.

2. Materials and methods

2.1. Fungal isolate

The isolate FDWSRU #78-03 of *P. carduorum* was used for field and greenhouse tests with *Cirsium* species. It was collected in 1978 by Emge, R.G., 29 km south of Ankara, Turkey. Based on data from the original evaluation, it was considered more aggressive than other isolates of *P. carduorum* from musk thistle. It also was the isolate recommended in the original proposal and approved for field tests in Virginia (Baudoin et al., 1993). Subsequently, *P. carduorum* was reported to have established in Maryland (Baudoin and Bruckart, 1996).

Another isolate (FDWSRU #98-103) was used for final greenhouse artichoke inoculations. It was collected near Mt. Shasta, California by Woods, D.M., CDFA. This isolate was likely the result of natural spread from the original study in Virginia to California, although this cannot be confirmed. It was used for evaluations on artichokes, because there is a significant artichoke industry in California that would be most likely exposed to this isolate. Also, if final approval for use of *P. carduorum* is granted for California, this is the isolate most likely to be used in distribution and dissemination programs.

2.2. Plants tested

In every study, healthy (noninoculated) musk thistle plants were included as susceptible controls. Species of *Cirsium* tested were: *Cirsium hydrophilum* (Greene) Jepson var. *hydrophilum*, *C. h.* var. *vaseyi* (Gray) Howell, J.T., *Cirsium fontinale* (Greene) Jepson var. *campylon* (H. Sharsm.) Keil and Turner, C., *C. f.* var. *fontinale*, *Cirsium loncholepis* Petrak, *Cirsium rhothophilum* Blake, and *Cirsium vinaceum* Woot. & Standl. Source of seed and the environmental concern for each species is given in Table 1. All are considered to be rare or endangered except *C. vinaceum*, which is listed federally as "Threatened" (US Fish & Wildlife Service, 2000). Recommendation was made to test *C. f.* var. *obispoense* Howell, J.T., as well, but viable seeds could not be obtained.

Four cultivars of artichoke, 'F1 (24),' 'Imperial Star,' 'DG-B,' and 'Cardone,' were tested. These were obtained from commercial artichoke seed suppliers or producers in California by Woods, D.M., CDFA, Sacramento, CA.

2.3. Field evaluation of Cirsium spp.

A field experiment was conducted over a 2-year period at the United States Department of Agriculture, Agricultural Research Service (USDA-ARS) facility in Frederick, MD. Experiments were designed to test field susceptibility of both E/T *Cirsium* spp. and musk thistle after natural spread of the pathogen. Three circular field plots were set out the first year, each with 48 test plants arranged 1–2 m from a cluster of infected musk thistle plants in the center (Fig. 1). Plots were set up with misters on risers to provide free moisture for the infection to proceed in the event of dry or drought conditions. The misters were never used since there was very good moisture over the two years of field evaluations. Grass was maintained between the infected musk thistle source plants and the test plants to reduce heat stress and drying conditions during the study.

Musk thistle source plants for *P. carduorum* in the field study were inoculated for the first year of tests on June 24, 1996. Inoculum was applied at the rate of 0.1 mg urediniospores per plant suspended in water with 0.125% Tween 20 (polyoxyethylenesorbitan monolaurate)

Table 1
Cirsium species tested in this study

Scientific name	Common name	Listing source[a]	Plant listing status[b]	Location of plant in US
C. fontinale				
var. *campylon*	Mt. Hamilton thistle	CNPS	1B,2-2-3	California
var. *fontinale*	Fountain thistle	F&WS	1B, E	California
C. hydrophilum				
var. *hydrophilum*	Suisan thistle	F&WS	1B, E	California
var. *vaseyi*	Vasey's thistle	CNPS	1B,3-2-3	California
C. loncholepis	La Graciosa thistle	F&WS	1B, E	California
C. rhothphilum	Surf thistle	CNPS	1B,2-2-3	California
C. vinaceum	Sacramento Mt. thistle	F&WS	T	New Mexico

[a] Sources: CNPS, California Native Plant Society, Inventory, Sixth Ed. (http://www.northcoast.com/~cnps/cgi-bin/cnps/sensinv.cgi). F&WS, US Department of the Interior, Fish & Wildlife Service, List of Threatened and Endangered Species, updated May 12, 2001 (http://ecos.fws.gov/tess_public/TESSWebpage).

[b] Status: 1B, 2-2-3 = (designations in sequence) "1B" means plant is rare, threatened, or endangered in California; "2" means it is distributed in a limited number of occurrences; "2" means it is endangered in a portion of its range; and " 3" means it is endemic in California. 1B, 3-2-3 = the same as the preceding, except the first "3" means the plant is distributed in one to several highly restricted occurrences, or present in such small numbers that it is seldom reported. E, Listed federally as Endangered. T, Listed federally as Threatened.

Fig. 1. Close-up of one plot; a, musk thistle source plants infected with *Puccinia carduorum*; b, musk thistle test plants among test plants of *Cirsium* spp. in the ring; and c, water line with risers to provide mist (i.e., artificial dew and irrigation) during the summer.

wetting agent. Plants were misted for 5 s every half hour through the night, and incubated in a separate greenhouse from the test plants. Infected rosettes were transplanted to the center of the plots on July 5, 1996, prior to pustule eruption when flecks were evident. Source plants were not used the second year (1997); infections resulted from naturally available inoculum in the field.

Seeds of test plants, including musk thistle, were allowed to imbibe overnight on filter paper saturated with gibberellic acid (GA_3, 10^{-3} M), planted in vermiculite, and transplanted into a pasteurized soil mix in 10 cm diameter clay pots on June 5 and 20, 1996. Test plants were set out in the field on July 5 and 6, 1996, before pustules erupted on the source plants. Individual plants were hand-watered when they were set and again on July 7 to insure good plant establishment.

In 1997, test plants were grown as described and transplanted to the field on June 27. The plot that had been harvested in 1996 was replanted at that time. For the two plots left to overwinter, only musk thistle ($n = 18$), *C. vinaceum* ($n = 15$), and *C. hydrophyllum* var. *vaseyi* ($n = 1$) plants survived the winter, so test plants of the other species for which seeds were available were used to fill the remaining spaces. None of the test plants had symptoms of infection when set in the field. Species used during the second season are given in Table 2. Plots were weeded as needed and the insecticide "Malathion 50" (diethyl[dimethoxythiophosphorylthio] succinate; 50% EC, applied at the rate of 0.23% a.i., Miller Chem. and Fertilizer, Pratt Gabriel Division, Hanover, PA) was applied twice in July 1997 after transplanting to control the defoliating shield beetle, *Cassida rubiginosa* Müller (Coleoptera, Chrysomelidae). Severely damaged or dead plants were replaced until the middle of July when either the supply of seedlings was depleted or the season had advanced to the point that such actions were not practical; i.e., plants would be too small for the test.

Ideally, there should have been six plants of each test plant accession in each plot, but some species were limited in number because of poor germination or limited seed supply, particularly in 1997. Seedlings that did not survive transplanting were replaced, usually with plants of the same species. The number of test plants for each species in each year is given in Table 2.

Plots were observed at least weekly the first year, and data for plant size, number of leaves, and number of infected leaves, were taken 1 and 2 months after transplanting (August 8, and September 10, 1996, respectively). On October 30, 1996, plants in one plot were removed and carefully examined for infection. Data on the number of plants infected during 1996 are presented in Table 2.

Two plots not harvested in 1996 were left to overwinter. Disease developed naturally the next spring from inoculum in the field; no artificial inoculations were made to these plots in 1997. Plants that survived the winter were examined on June 19, 1997, for rust infection. Plants were

Table 2
Cirsium spp. and *Carduus nutans* ssp. *leiophyllus* in three field plots naturally infected by *Puccinia carduorum* in 1996 and 1997 field evaluations[a]

Species	1996			1997	
	August 8	September 10	October 30[b]	June 19[c]	October 22
Cirsium					
fontinale					
var. *campylon*	0/10[d]	0/10	0/6	—[e]	0/23
var. *fontinale*	0/15	0/15	0/2	—	0/4
hydrophilum					
var. *hydrophilum*	0/6	0/6	0/5	—	0/28
var. *vaseyi*	0/25	0/25	0/8	0/1	NT[f]
loncholepis	0/24	0/24	0/2	—	
rhothophilum	0/11	0/11	0/4	—	NT
vinaceum	0/25	0/25	0/9	0/15	0/10[g]
Carduus					
nutans ssp. *leiophyllus*	19/27	26/26[g]	8/8	18/18	14/16[g]

[a] Data for the three plots combined.
[b] Data from one of three plots destructively sampled and examined carefully for infections.
[c] Data from plants that survived through the winter in the two plots not destructively sampled in 1996.
[d] Number of plants infected/total number of plants.
[e] "—," Plants that did not overwinter.
[f] NT, Not tested.
[g] Some plants were lost for reasons other than the rust disease.

rated for maximum pustule size using a scale of 0–4, where 0, no infection; 1, very small pustules (<0.30 mm diam, a very resistant reaction); 2, small pustules possibly with some chlorosis (a resistant reaction); 3, medium pustules and good spore production (a susceptible reaction); and 4, large pustules with very good sporulation and development of secondary (satellite) pustules around "parent" infections ($\geqslant$0.60 mm diam, a very susceptible reaction). Data were analyzed with the Median option of PROC NPAR1WAY, using SAS software (SAS Inst., Cary, NC).

One week after rating the winter survivors for disease, the plots were re-established on June 27 using transplants of accessions to fill spaces not occupied by *C. vinaceum*, *C. hydrophilum* var. *hydrophilum*, or *C. nutans*. Plots were monitored and managed as described above. Final data were collected on October 22, 1997 for the number of plants, number of leaves, and infection, based ratings for proportion of plant infected (0–4; 0, no infection; and 1, 2, 3, 4 = <6%, 6–10%, 11–30%, and >30% of the leaves with pustules, respectively). Means and confidence intervals ($P = 0.05$) were calculated for leaf counts, and rating data were evaluated using the Median option of PROC NPAR1WAY in SAS.

2.4. Greenhouse evaluations

Test plants (including musk thistle) were grown from seed in the USDA-ARS plant pathogen containment greenhouse facility at Frederick, MD (Melching et al., 1983). After five weeks, plants were inoculated with *P. carduorum* isolate 78–03. Test plants and musk thistle were sprayed with a suspension of urediniospores (0.1 mg per plant) in water plus 0.125% Tween 20, given a 16 h dew at 18–20 °C, allowed to dry in indirect light, and then placed in a greenhouse with natural light and temperatures maintained between 22 and 25 °C. Inoculated plants were monitored for symptom development.

Two separate inoculations were made for tests with *Cirsium* spp. The species and numbers of individuals (given in Table 3) varied according to availability of seed. Pustule counts were made two weeks after inoculation on the three most-infected leaves from each rosette. Plants, particularly the *Cirsium* species, were observed for at least two additional weeks to assure that no infections developed. Data were subjected to analysis of variance (PROC GLM) and mean separation using predetermined comparisons of predicted levels of significance (the PDIFF option in GLM) using SAS. Differences were considered significant at $P \leqslant 0.05$.

Artichoke plants and musk thistle were inoculated either 4 or 9 weeks after seeding. Three inoculations of artichoke and musk thistle were made. The number of plants inoculated for each cultivar in two representative replications is given in Table 4. In all the experiments, pustules were rated for maximum size on any individual plant using a scale of 0–4, described above. A 10× magnifier was used to inspect artichoke leaves, because pustules often were very small and difficult to see with the unaided eye. In addition to pustule counts, leaf area was measured using a Lamda Area Meter (LI-COR, Lincoln, NE) in the final two experiments. Data were collected either 2 or 4 weeks after inoculation for total number of pustules and the number of pustules per square centimeter of leaf and were analyzed as described, using PROC GLM and the PDIFF option for mean separation in SAS. Data on the proportion of plants in each rating category and pustule density from the last two experiments are presented as representative.

Table 3
Least square means for pustules per plant and pustules per cm^2 on *Cirsium* spp. and *Carduus nutans* ssp. *leiophyllus* from inoculations with *Puccinia carduorum* in a greenhouse

Species	Experiment 1			Experiment 2		
	Plants[a]	Pustules[b]	PLA[c]	Plants[a]	Pustules[b]	PLA[c]
Cirsium						
fontinale						
var. *campylon*	20	0 b	0 b	20	0 b	0 b
var. *fontinale*	20	0 b	0 b	20	0 b	0 b
hydrophilum						
var. *hydrophilum*	3	0 b	0 b	5	0 b	0 b
var. *vaseyi*	10	0 b	0 b	15	0 b	0 b
loncholepis	NT[d]	—	—	1	0 b	0 b
rhothophilum	10	0 b	0 b	18	0 b	0 b
vinaceum	7	0 b	0 b	NT	—	—
Carduus						
nutans ssp. *leiophyllus*	40	58.9 a	1. 58 a	20	30.3 a	1.23

[a] Number of plants examined.
[b] LSMEANS from the GLM Procedure of SAS (SAS Inst., Cary, NC) for mean number of pustules per plant, based on average pustule counts from the three most-infected leaves. Means followed by the same letter in each column are not significantly different, $P = 0.05$.
[c] PLA, Pustules per unit Leaf Area; LSMEANS of pustules per cm^2 of leaf tissue. Means followed by the same letter in each column are not significantly different, $P = 0.05$.
[d] NT, Not tested.

Table 4
Susceptibility of four commercial cultivars of *Cynara scolymus* (artichoke) and *Carduus nutans* ssp. *leiophyllus* (musk thistle) to *Puccinia carduorum* form California[a]

Test plant	Cultivar	n	Pustule type[b]					PLA[c]
			0	1	2	3	4	
Artichoke	F1 (24)	32	43.8[d]	53.1	3.1	—	—	0.34 a
Artichoke	Imp. Star	30	86.7	13.3	—	—	—	0.10 a
Artichoke	DG-B	33	60.6	39.4	—	—	—	0.10 a
Artichoke	Cardone	20	45.0	50.0	5.0	—	—	0.10 a
Musk thistle		22	—	—	—	4.6	95.4	3.51 b

[a] Data are combined from two replications.
[b] Pustule type: plants were rated for maximum pustule size using a scale from 0 to 4, where: 0, no infection; 1, very small pustules (<0.30 mm diam, a very resistant reaction); 2, small pustules possibly with some chlorosis (a resistant reaction); 3, medium pustules and good spore production (a susceptible reaction); and 4, large pustules with very good sporulation and development of secondary (satellite) pustules around "parent" infections ($\geqslant$0.60 mm diam, a very susceptible reaction).
[c] PLA, LSMEANS of pustules per cm^2 of leaf tissue. Means followed by the same letter in each column are not significantly different, $P = 0.05$.
[d] Percent of individual plants for each pustule type.

3. Results

3.1. Cirsium evaluations

Only musk thistle plants (*C. nutans* ssp. *leiophyllus*) were infected by *P. carduorum*. In the field, 70% of the musk thistle test plants (19 of 27 tested) had symptoms of infection by August 8, 1996 (Table 2). By September 10, 1996, disease incidence for musk thistle in all plots was 100% (Table 2) and an average of 37% of the leaves on each plant was infected. Representatives of each *Cirsium* species were considered to be of good quality for this test. Data from the single plot harvested in October 1996 (Table 2) confirmed findings from earlier ratings (Bruckart et al., 1996a). All musk thistle plants ($n = 8$) were infected and an average of 71.3 ($\pm$14.5)% of the leaves had pustules. Good infection was noted on several musk thistle leaves (Fig. 2).

The two plots left to overwinter were re-examined in the spring of 1997. Only *C. hydrophyllum* var. *vaseyi* ($n = 1$), *C. vinaceum* ($n = 15$), and musk thistle ($n = 18$) survived the winter. The June 19 ratings indicate only musk thistle was infected by *P. carduorum* (Table 2). Ratings made on October 22, 1997 also showed that only musk thistle was susceptible to *P. carduorum* (Table 2). Ratings for the number of infected leaves for disease were low (average 0.94 ± 0.22), indicating less than 6% of the leaves had pustules. This was most likely due to the warmer and drier conditions occurring after July 1997 in Maryland. Nonetheless, 87.5% of the individual musk thistle plants were infected at the time of the final rating.

Greenhouse inoculations confirmed results from the field (Table 3). There was an average of 40.8 and 30.3 pustules per leaf on musk thistle in each of two experiments, respectively; and no infection was noted on any of the *Cirsium* test plants (Table 3).

3.2. Artichoke evaluations

Artichoke plants from each of the four cultivars were infected under optimal greenhouse conditions; disease incidence was 40% (46/115). All but two infected plants (1.7%) were rated '1,' having very resistant reactions

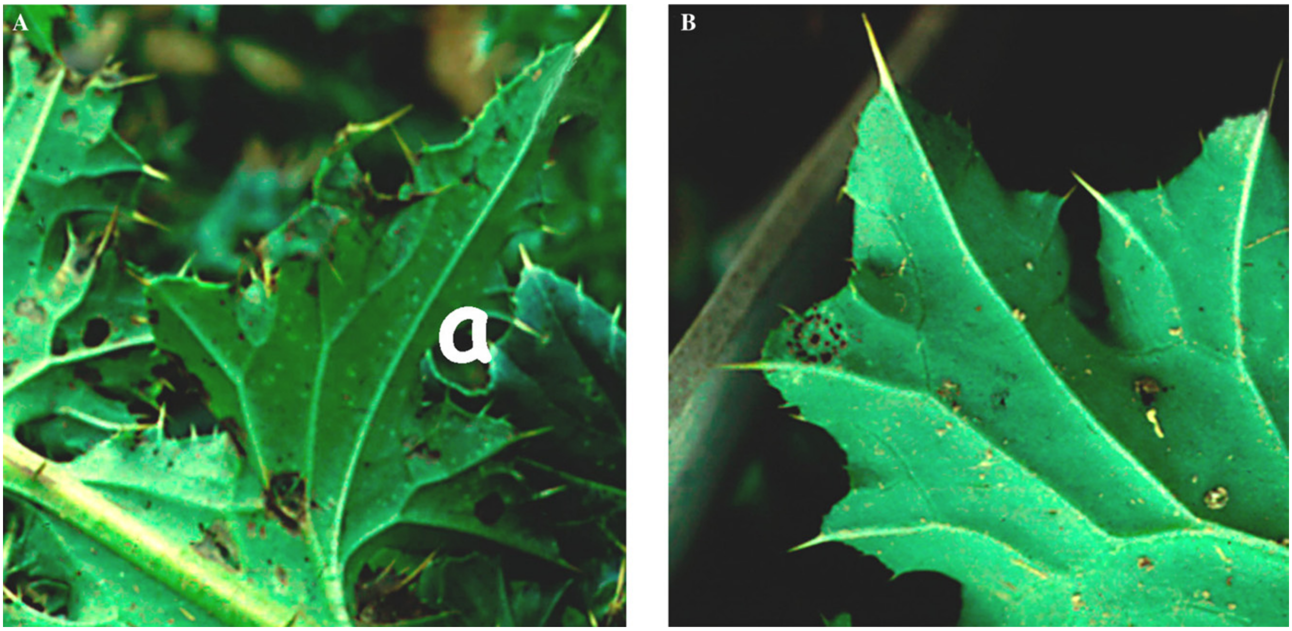

Fig. 2. Symptoms on musk thistles in the field in 1996. (A) Pustules and flecks; a, leaf with several flecks. (B) A large pustule with satellite pustules.

with very small pustules (⩽0.30 mm diam). The remaining two plants were rated ‘2,’ a resistant reaction with slightly larger pustules. Pustules on infected artichoke plants were fewer in density than those on musk thistle (Table 4, Fig. 3), and findings were similar to those from a preliminary inoculation in this study that did not

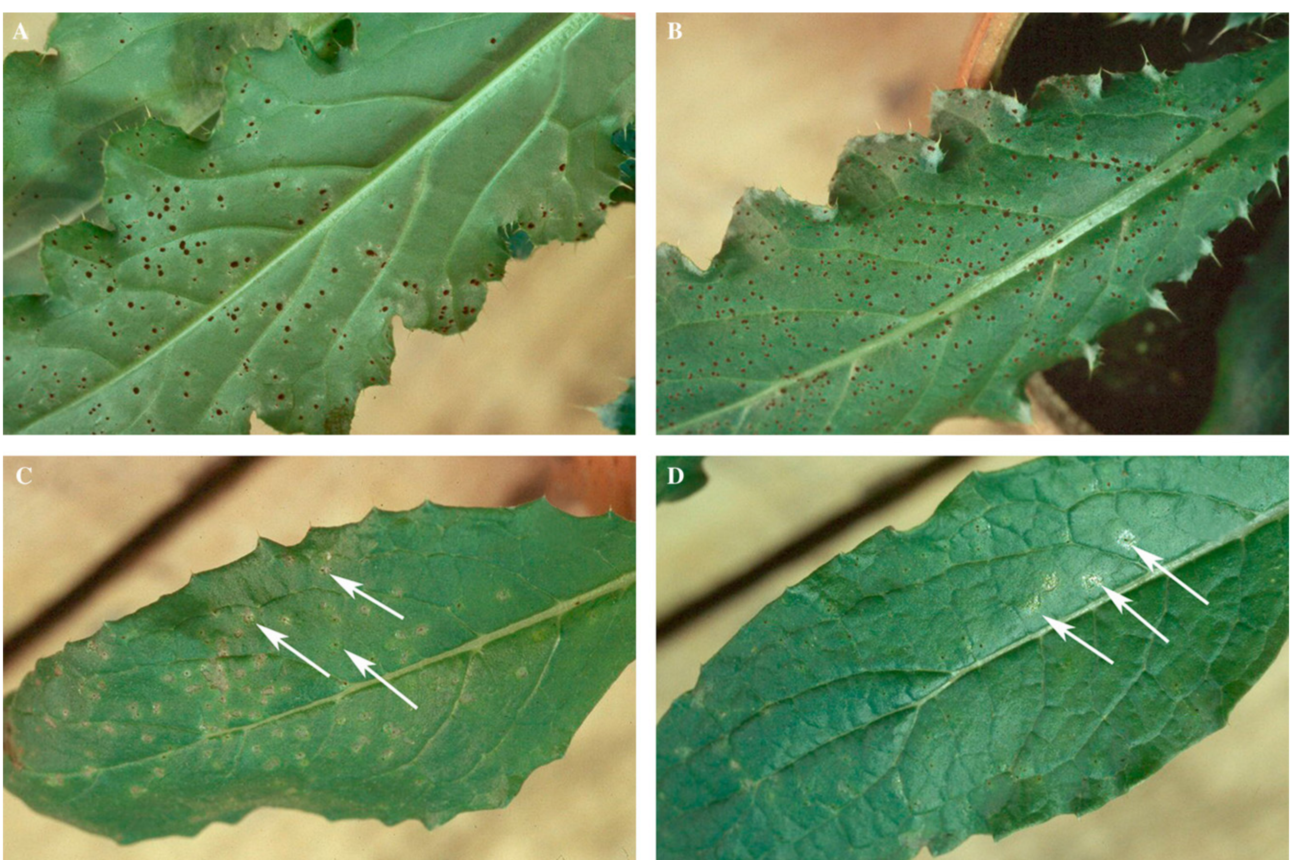

Fig. 3. (A,B) Typical symptoms of infections on musk thistle in the greenhouse. Pustules with satellite flecks (A). (C,D) Most infection (worst-case scenario) noted on artichoke; arrows point to typical pustules rated “1” in the 0–4 scheme for pustule size.

include leaf area measurements. The greatest proportion (60%) of artichoke plants was not infected (rating of "0"; Table 4). Pustule counts on some individual leaves of artichoke were high, but pustules were always small (Table 4, Fig. 3) and overall numbers of pustules per unit leaf area (PLA) in populations tested were very low (Table 4). Ratings on musk thistle in the artichoke studies indicate all individuals were either "susceptible" (rated "3") or very susceptible (rated "4"; Table 4, Figs. 3A and B), and pustule density was at least 10 times greater on musk thistle than on artichoke (Table 4).

4. Discussion

The conclusion from earlier studies that *Cirsium* species are not good hosts of the musk thistle isolate of *P. carduorum* (Baudoin et al., 1993; Bruckart et al., 1996a; Politis et al., 1984) is further substantiated by the data from these tests. None of seven rare or E/T species of *Cirsium* developed disease symptoms after natural exposure to the pathogen in the field or from direct inoculation in greenhouse tests. Musk thistle test plants were readily infected by the fungus in all experiments regardless of the inoculation process. Similar results occurred in an earlier field study in Virginia (Baudoin et al., 1993); the exposure of other *Cirsium* spp. and artichoke resulted in only a single, small pustule (a very resistant reaction) on one of 32 artichoke plants in the three years of the test (Baudoin et al., 1993). As in this study, musk thistle test plants in the previous study were readily infected after natural spread of the pathogen.

The new, untested artichoke cultivars were infected in greenhouse tests only after direct inoculation with *P. carduorum* under conditions highly favorable for disease. These results were not unexpected and were similar to reactions described previously (Bruckart et al., 1996a; Politis et al., 1984). Artichoke was clearly resistant in this and previous studies. In this study, 60% of the individual plants were symptomless, and 98.3% of all inoculated individuals were rated either "0" or "1." As in the earlier studies, there was never enough inoculum from infected individual artichoke plants to reinoculate healthy plants, even under optimal greenhouse conditions. The conclusion is that the new artichoke cultivars will not likely be infected, much less damaged, by *P. carduorum* under field conditions. This is based on considerations that greenhouse reactions in this study were similar to those noted in earlier greenhouse tests and that field results in Virginia clearly showed a lack of disease from natural levels of inoculum in three years of tests (Baudoin et al., 1993). Furthermore, a strain of *P. carduorum* from *Carduus tenuiflorus* W. Curtis in California growing near artichokes (Watson and Brunetti, 1984), also caused infection of artichoke under ideal greenhouse conditions (Bruckart and Peterson, 1991). The California strain of *P. carduorum* has not been reported on artichoke.

Data from this study were used in two reports, the first to the F&WS, through APHIS, in 1996, and the other to representatives of the artichoke industry in 2001. Following submission of the report concerning studies with *Cirsium* spp., a letter was sent from APHIS to the F&WS requesting concurrence that, "*P. carduorum* is not likely to adversely affect listed [*Cirsium*] species, and is not likely to jeopardize the continued existence of proposed species …" (Letter from the Deputy Director of the USDA, APHIS, Environmental Analysis and Documentation, Policy and Program Development, dated August 12, 1997). After review, the F&WS concurred with the APHIS decision (Letter from the Acting Regional Director, F&WS Region 1, dated May 7, 1998). The report about artichoke results was provided to artichoke industry representatives through the CDFA, and no response from industry representatives was interpreted as a lack of concern on their part about the proposed action.

Based on concurrence from the F&WS and interpretation of the artichoke industry position, regulators with CDFA submitted a PPQ Form 526 (Application and Permit to Move Live Plant Pests and Noxious Weeds) in May 2002 to the USDA-APHIS with a request for permission to include *P. carduorum* in biological control programs against musk thistle. Final approval is pending. This action was taken, despite the fact that *P. carduorum* was likely spreading westward from its original release site in Virginia (Baudoin and Bruckart, 1996). Subsequently, it was discovered in Oklahoma (Littlefield et al., 1998) and California (Woods et al., 2002). The permit request has been pursued, even in light of these discoveries, to bring closure to the permit process, to enable CDFA personnel and others to legally deploy the rust where needed, and to facilitate approval of other isolates of *P. carduorum*.

This research concludes the risk analysis of *P. carduorum* for biological control of musk thistle. Throughout this process, a scientific research approach has been taken to identify potential hazards, measure risk, and to enable those in authority to make confident decisions about permitting introductions of foreign candidate agents (Bruckart and Shishkoff, 1993; Bruckart et al., 1996b). For susceptibility of both artichokes and native *Cirsium* spp., studies were conducted on the relative amounts of disease, both in the greenhouse (Bruckart and Dowler, 1986; Bruckart and Peterson, 1991; Bruckart et al., 1996a) and in the field (Baudoin et al., 1993; Bruckart et al., 1996a). Throughout the process, attempts have been made to keep regulators and interested parties, particularly artichoke growers and those charged with protecting the environment (e.g., F&WS), informed about accomplishments and plans.

One outcome from the risk analysis of *P. carduorum* and proposal for release is a process that is much better defined for review and permit decisions regarding the introduction of foreign plant pathogens as classical biological control agents. The important role of regulators has been clarified and improved. This is particularly true for the part played by the Technical Advisory Group on Biological Control of Weeds (TAG) and for the role of the F&WS. Even so, the process could be further improved, thus facilitating plant pathologists to contribute fully to the integration of plant diseases into weed management programs.

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